ON THE ETIOLOGY OF ACNE VULGARIS AND ITS TREATMENT BY VACCINES

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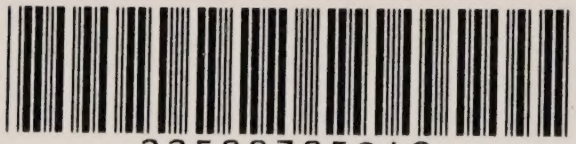
Reprinted from THE LANCET, April 10, 1909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ON THE ETIOLOGY OF ACNE VULGARIS AND ITS TREATMENT BY VACCINES.

An accurate conception of the etiological factors in the various bacterial diseases has of late years become increasingly important in view of the specific treatment of such diseases by means of vaccines. I here propose to set forth some investigations I have carried out on the bacteriology of acne vulgaris, which seem to show that this condition is not simply one of staphylococcic infection, but is largely due to a specific bacillary infection.

In 1893 Unna[1] first described a bacillus which he found in the lesions of acne vulgaris and to which he gave the name "acne bacillus." He did not isolate the bacillus in culture but found it constantly in sections and smears of comedones and pustules, and he concluded on histological grounds that the bacillus described by him was the probable cause not only of the comedo but also of the pustule. His three chief reasons for this belief were (1) that the bacillus was the only organism constantly present; (2) that in the comedo the bacilli were situated especially in the deeper part, whereas the other organisms present—e.g., staphylococci or bottle bacilli—were found near the surface; and (3) that the histological characters of the pustules were not such as were associated with other staphylococcic infections.

Sabouraud,[2] in 1897, described the bacillus as occurring in sebaceous plugs which can be squeezed out of seborrhœic skins. He claimed that it was the cause of oily seborrhœa and also of alopecia areata, which he considered a localised seborrhœic condition. He was the first to describe accurately

[1] Histopathology of the Skin.

[2] Annales de l'Institut Pasteur, 1897; Séborrhée, Acne, Calvitie, Paris, 1902.

the cultural properties of the microbe, which he named the "bacille de séborrhée grasse." He considered that the indurated nodules and the pustulation of acne were due to a superimposed secondary infection with staphylococci.

Gilchrist,[3] in 1899 and later in 1903, states that a bacillus which is apparently identical with that described by Unna and Sabouraud is the cause of all the lesions of acne. He recounts that he found the bacillus in all the smears of acne pustules, and in about 30 per cent. of cases he obtained pure cultures from these pustular lesions. He rechristened the organism the "bacillus acnes."

Whitfield,[4] in his book on skin diseases, concludes as the result of his investigations that the acne bacillus is the cause of the comedo, but that the pustule is the result of a secondary staphylococcic infection.

The acne bacillus, as seen in smears of comedones and pustules, is a small Gram-staining bacillus varying in length from under 1μ to 3μ or 4μ and about $\frac{1}{2}\mu$ wide. The younger forms stain evenly and darkly, but commonly the older forms show one or more deeply stained dots in their interior. These dots may be situated at the poles or they may be present along the whole length, resembling a chain of small cocci. The tenacity with which these bacilli retain Gram's stain is not nearly so marked as in the case of the staphylococcus and it is quite easy to so stain a film that the bacilli are decolourised, while the cocci are deeply stained. The arrangement of the bacilli in pus is very irregular, single bacilli are present, pairs are common, especially arranged as a V, and large irregular groups are almost invariably to be seen; the microscopical characters of the bacillus in pus films and also in smears from cultures are very similar to some of the diphtheroid bacilli one meets with in superficial ulcers and elsewhere, and it is very probable that it is to this group that the bacillus belongs. The bacilli are often found inside the pus cells, and the same film may show phagocytosis of both bacilli and cocci, indeed one may see both taken up by the same cell. In the comedo the bacilli are in general shorter and stouter, and stain evenly, while in the pustule longer forms are seen with very irregular staining.

Smears from acne pustules practically always show the

[3] Johns Hopkins Hospital Reports, vol. ix.; Journal of Cutaneous Diseases, 1903.

[4] Skin Diseases and their Treatment, 1907.

presence of the specific bacillus. Often no other organism is to be found, but in many cases staphylococci are evident in greater or less numbers. Other organisms may be seen, but they are not common and cannot have much influence on the etiology of the lesion. Gilchrist found the bacilli in every one of his smears, and in only very few have I been unable to find them with the greatest ease. In six cases, however, I was unable to find any organism at all, but from two of these I was able to obtain pure cultures of the bacillus. I found only acne bacilli in 44 per cent. of the pus films I examined, acne bacilli with staphylococcus in 53 per cent., in six cases no organisms were observed, while in one case,[5] only bottle bacilli were evident. The films were stained in all cases by Gram's method, which is much the best for the demonstration of the organisms. The bacilli in most cases were very numerous and the cocci when present were usually relatively few in numbers, but in some cases (roughly about a third of those cases where both organisms were present) the cocci were very numerous and far outnumbered the bacilli.

Unfortunately the very slight power of growth of the acne bacillus on artificial media makes it very difficult to get from cultural methods a true estimate of the frequency of its occurrence. Unna, when he described the organism, had not obtained cultures, and Sabouraud cultivated it from comedones and seborrhœic lesions. Gilchrist, however, claims to have cultivated it from acne pustules in 96 cases out of 145, 44 times mixed with staphylococcus and 52 times pure. My success in obtaining pure cultures has not been so great as was Gilchrist's, for out of 132 cultures from acne pustules I could only get pure growths of the bacillus on 13 occasions. Of the other cultures 35 were sterile, 40 grew both acne bacillus and staphylococcus, and 44 grew only staphylococcus. The large number of sterile cultures is due, I believe, to the fact that the bacillus only grew with the greatest difficulty on some of the media used. In some of the cultures, although staphylococci grew yet so few colonies developed that it suggested an accidental contamination, especially as it was impracticable to efficiently sterilise the skin in some cases. The sterilisation of the skin was effected by wiping it over with pure lysol and washing this off quickly with spirit. The pustule was

[5] A man, aged 48 years, who had had comedones and pustules on the back for only about two years.

then pricked and the contents squeezed out and a loopful transferred to an acid agar slope and planted out *en masse*. If the pus be spread thinly over the surface of the medium no growth of the bacillus will be obtained. The same applies to subculturing the bacillus and many tubes where it is only moderately thickly planted fail to grow.

In the large deep-seated pustules two elements are to be observed in the pus. Where the contents are squeezed out, first appears an opaque semi-fluid mass, consisting chiefly of pus cells. If this is wiped away and further pressure is made a gelatinous mass is extruded consisting largely of epithelial cells and representing probably a broken-down comedo. In making my cultures I have preferred to use the purulent material for two reasons: firstly, because in investigating the causation of the pustules it seemed hardly fair to use for cultural purposes what I considered to be a degenerated comedo, and secondly, in those cases where I examined films of both constituents of the pustule I found that where staphylococci were present in the pus they were present also in the gelatinous substance. A large number of my cultures were taken also from the small superficial pustules, and these were the more productive in pure cultures of the bacillus.

The subordinate rôle played by staphylococci in some cases of acne was well illustrated in one patient who suffered from comedones and pustules on the back and shoulders. Films of these pustules I have examined over 50 times and only on a few occasions could I find any cocci present, while of eight cultures on acid agar five yielded pure cultures of the acne bacillus, two were sterile, and staphylococcus grew in only one. Of eight cultures made on ordinary agar six were sterile and on the other two a few colonies only of staphylococcus developed.

In cases where a pure culture of the bacillus was obtained no change was observed in the culture tube for about three days but an increase in the transparency of the mass of pus. Then three or four small white spots appear, representing colonies of the bacillus, and these increase slowly in size till at the end of a fortnight they may have the diameter of 1 millimetre. The fact that only three or four colonies appear from a large loopful of pus indicates the feeble character of the growth, as films of these cases have shown that a very large number of bacilli must have been present.

It has been suggested that the reason of frequent failure

to cultivate staphylococcus from an acne pustule is that in acne, as in some other coccal infections, the cocci are limited to the walls of the abscess, but this does not, I think, apply to acne, as sections reveal that in this condition the cocci are more or less scattered throughout the pus, so that when one takes a large quantity of pus it is hardly conceivable that they should be missed.

In culture the bacillus presents exactly the same appearance to that already described as being observed in pus films. Involution forms are not prominent and it retains its staining properties in a culture three months old. Definite branching forms occur, especially in broth cultures. It does not form spores. It grows best at 37° C. and growth ceases at room temperature. Sabouraud gives the thermal death point as 75° C., and he has taken advantage of this to separate it from other organisms. Only once have I been able to separate it from staphylococcus by heating to 60° for half an hour. On the other occasions I have tried this method I was unable to obtain any growth after heating. Other methods of separation have been recommended, such as soaking comedones in absolute alcohol when the cocci only are said to be killed or leaving the culture for three months when the cocci are said to have died out, but with these I have had no success. The cultural characteristics are as follows :—

Broth.—This becomes cloudy in two days, and on the sides and bottom of the tube a heavy flocculent deposit is produced. When growth ceases in about a fortnight the fluid is quite clear with a heavy deposit at the bottom. The broth is not rendered acid by the growth.

Acid agar (ordinary agar rendered faintly acid with HCl). —On the third day a small rounded colony appears, increasing slowly in size for a fortnight, when it may measure up to one millimetre. The colony remains white and much resembles that of staphylococcus albus.

Acid glycerine agar.—The growth is similar to that on acid agar but not quite so rapid. When old it becomes heaped up and darkens in colour till it is almost black.[6]

[6] This darkening on glycerine media may be due to the formation of the same substances which cause the blackening of the free end of the comedo.

Sabouraud's special medium.—I have found the rate of growth on this much slower than on either of the two preceding solid media, but the type of growth is the same. On ordinary agar no growth occurs.[7] No growth was obtained in broth containing 1 per cent. of the various sugars.

Oleic acid glycerin agar.—This medium was suggested to me by the constant association of the bacillus with the fatty secretion of the sebaceous glands. Agar is neutralised with hydrochloric acid, 2 per cent. glycerine and 0·1 per cent. oleic acid is added, and the medium is then tubed off and sterilised in the autoclave in the usual way. Subcultures on this medium showed an appreciable growth in 24 hours, and in two days the surface was covered with a mass of white opaque colonies resembling very much those of staphylococcus albus both in size and appearance. This is by far the most suitable medium I have tried for the bacillus.

We have now to examine the serum reactions of infected persons to the acne bacillus. Gilchrist found that the serum of his patients agglutinated the bacillus in a hundred-fold dilution while his controls of healthy serum only agglutinated in a fifty-fold dilution. These results I have been unable to reproduce in their entirety. The sera were tested with five strains of the bacillus and three of the sera were derived from the same individuals from whom three strains of bacillus had been isolated. Of these three one showed no agglutinating power, one agglutinated the homologous strain in an 18-fold dilution, and the other agglutinated its own bacillus in a 54-fold dilution, and at the same time showed agglutination with one of the other strains to a less degree (six-fold dilution). Two patients who were being inoculated with a certain strain of bacillus and improving rapidly agglutinated this strain in a 32-fold dilution. The normal sera showed no agglutinating power in a four-fold dilution.

As regards the opsonic power I have tested 26 patients and have found that the opsonic index varies considerably, much more than does that of serum from healthy people. The

[7] Since writing the above I have succeeded in obtaining from the contents of a superficial culture planted on ordinary agar after 14 days' incubation four small colonies of bacilli presenting appearances similar in all respects to the acne bacillus.

indices obtained varied about the normal in the following manner:—

From 0·60 to 0·80 = 5	From 1·21 to 1·40 = 5
,, 0·81 ,, 1·00 = 6	,, 1·40 ,, 1·60 = 6
,, 1·01 ,, 1·20 = 4	

Thus it will be seen that there is a tendency to an increase in the production of opsonin in infected persons—a condition frequently met with in other infections—e.g., gonococcus, bacillus coli, &c.

The opsonic indices of seven apparently healthy people were done in all 42 times, and these may be tabulated as follows:—

Under 0·80 = 1	1·01 to 1·10 = 15
0·81 to 0·90 = 6	1·11 ,, 1·20 = 6
0·91 ,, 1·00 = 13	Over 1·20 = 1

As regards the pathogenicity of the bacillus to the lower animals Gilchrist found that it killed mice and guinea-pigs in one or two weeks and from the pus at the seat of inoculation and from the heart's blood he obtained pure cultures of the bacillus. My observations in this connexion are limited to the inoculation of week-old broth cultures of two strains of bacillus into two-guinea pigs, neither of which suffered any inconvenience.

The next question to be examined was the possibility or otherwise of reproducing the disease by inoculation of the bacillus directly into the skin of a susceptible individual. Last November I rubbed vigorously into the skin of the forearm of a person suffering from acne a week-old broth culture of an acne bacillus freshly isolated from him. At the same time the same culture was vigorously rubbed into the arm of one of my colleagues who has never suffered from acne. In the latter case only a negative result was obtained but in the case of the susceptible individual after two days several of the hair follicles of the area rubbed over became inflamed and in five days definite pustules formed which persisted a week, then began to dry up, although the lesions did not disappear for over a month. On the fifth day and again on the twelfth day films of these pustules were examined and no organisms other than the acne bacillus could be discovered. This, then, would seem to show that this bacillus is capable of producing a pustular folliculitis in a susceptible person.

Of the other organisms present in acne I need only mention two—viz., the staphylococcus and the bottle bacillus. The former is present in many comedones as well as in pustules and sometimes in considerable numbers ; indeed, Sabouraud in his earlier researches had great difficulty in obtaining culture from these comedones of the acne bacillus free from staphylococci. Staphylococcus aureus seems to be uncommon in these lesions. I have only grown it on two occasions, once from a large pustule—almost a furuncle—following an excessive dose of staphylococcus vaccine, and again in what seemed an ordinary slight case of pustular acne. All my other staphylococcic cultures were of the white variety and only very few of those tested liquefied gelatin. Of the others there were two main types—one very opaque and white, while the other was greyish semi-transparent, having a very flat colony with irregular margin which spreads to a considerable size over the agar.

The bottle bacillus is a large organism shaped something like a bottle and presenting an appearance very like a yeast. I have not succeeded in obtaining cultures. It occurs in the more superficial part of the comedo and is to be found constantly also in dry seborrhœic conditions of the scalp or elsewhere where its presence may be of great diagnostic value.

Before going on to the question of the treatment of acne I would like to direct attention to the evidence that the acne bacillus may be and frequently is the cause of pustulation. As stated above, Unna and Gilchrist thought that the pustulation was of bacillary origin, while Sabouraud, and more recently Whitfield, believed it to be a secondary coccal infection. The last observer gives as his reasons for holding to the coccal origin of the pustules (1) that staphylococci can always be cultivated from the pus; (2) that films of pus often show cocci ingested by leucocytes, thus proving that they are not merely a surface contamination ; and (3) that patients with pustular acne have a low opsonic index to staphylococcus. As regards the first reason the cultural evidence detailed in an earlier part of the paper would seem to indicate that there is a fairly large percentage of cases where one obtains either no growth at all from the pustules or a growth of the acne bacillus. This bacillus one can practically always see in films of pus and it is probably only because of its very feeble powers of reproduction on artificial media that one does not obtain a culture every time. With regard to the ingestion of the cocci by leucocytes

if this be a proof that they are exerting a pathogenic action, then much more does this apply to the acne bacillus, for in pus films one finds bacilli inside the pus cells with much greater frequency than one does cocci. Sometimes, as I indicated above, one finds both phagocyted in the same film or even both inside the same pus corpuscle. The third reason adduced—that of the low opsonic index—applies, I think, only to those individuals in whom there is a staphylococcic as well as a bacillary infection. I have shown, however, that these patients also exhibit abnormal opsonic indices to the acne bacillus.

Having now treated in a more or less complete manner of these objections I would adduce the following contentions in favour of the pyogenic nature of the bacillus. 1. It is constantly present in the pus as shown by examination of films. 2. It is frequently the only organism present in pus. 3. It can frequently be recovered from the pus in pure culture. 4. Inoculated into the skin of a susceptible individual it produced a pustular folliculitis and examination of the pus showed no other organism to be present. 5. Inoculated into animals it produces an abscess at the seat of inoculation from which the bacillus has been recovered in pure culture. (Gilchrist.) 6. The serum of patients affected with acne agglutinates the bacillus in some cases. 7. The opsonic indices of patients to the acne bacillus vary considerably from normal. 8. Large doses of a vaccine derived from the acne bacillus have, as we shall see later in connexion with the treatment, induced a marked negative phase characterised by the appearance of fresh pustules from which the bacillus has been recovered in pure culture. 9. A vaccine of this organism has a marked beneficial effect on the condition. 10. When a patient has a high opsonic index to the bacillus his condition improves, but when the opsonic index is low fresh pustules appear.

We now come to the question of the treatment of acne vulgaris by means of bacterial vaccines. I think we may take it is definitely proved now that in a localised infection, when one inoculates the patient with appropriate doses of a carefully prepared vaccine derived from the infecting organism, one obtains a definite beneficial effect. This has been shown by very many observers since Sir Almroth Wright first directed attention to the curative properties of these vaccines, and taking this broad principle as the basis for our work we would expect, from what I

have advanced as to the bacteriology of acne, that a vaccine of only one organism, whether it be staphylococcus or acne bacillus, would not be sufficient in all cases. The treatment of acne by means of staphylococcus vaccine has been repeatedly discussed by Sir Almroth Wright [8] and others, and I here propose to deal mainly with the treatment by means of a vaccine of the acne bacillus alone or in combination with staphylococcus.

Very frequently in treating a case of acne with staphylococcus vaccine it is found that there is a definite improvement for a time then the condition remains stationary in spite of any modification in the dose or character of the staphylococcic vaccine. This is exactly what would be expected from a consideration of the bacteriology, as it is only in a certain number of cases that the staphylococcus is present, and even when present it is not responsible for the whole condition. We can, therefore, divide cases of acne vulgaris for therapeutical purposes into three classes. The first will consist of those cases in which the comedo is the dominant feature, with, also, some of those which have progressed further to the indurated and pustular stages. In this class of case the acne bacillus is the offending agent and the staphylococcus if present at all is playing a subordinate rôle. This class, therefore, may be treated with a vaccine of acne bacillus alone. The second class consists of a large number of the indurated and pustular varieties where both staphylococcus and the acne bacillus appear to be playing an active part in the causation of the inflammatory process. The therapeutic requirements of this class are best met with a mixed vaccine of staphylococcus and acne bacillus. The third class consist of those less common cases where there is a more acute inflammation, tending almost towards a furuncular form. Here microscopical examination will show that the staphylococcus is the chief offender and treatment should be directed at first against this factor, not forgetting, however, that at the basis of the condition there is an acne bacillus infection.

I would here lay stress on the importance of the microscopical examination of smears of the pustules before vaccine treatment is embarked on in order that some certainty may be arrived at as to which class the patient belongs.

[8] The Lancet, March 29th, 1902, p. 874; Brit. Med. Jour., 1904.

The vaccine used was derived from a three weeks' old culture on acid agar and was made and standardised in the usual way. In two cases opsonic estimations were made almost daily over a period of six weeks, and the charts show that the index varied exactly as it does with inoculations of other organisms One of these charts relating to a case of mild pustular acne is here reproduced. A dose of 10,000,000 acne bacilli caused a marked negative phase followed by a rise and a subsequent drop. Smaller doses of 6,000,000 showed no negative phase. The clinical condition showed a very marked correlation with the index—i.e., when there was a low index fresh spots appeared and when the index rose the condition improved. This very close correlation rendered the continuance of the opsonic estimations unnecessary for guidance in treatment, the clinical condition affording a good and much more easily observed guide. I may mention here that this patient, who exhibited a marked negative phase with 10,000,000 bacilli, had previously had administered a dose of 50,000,000 which was followed by a very luxuriant crop of pustules, and from these pustules pure cultures of the acne bacillus were obtained.

The stock vaccine in use in this laboratory was derived from a pustule on the face of a woman, aged about 25 years, who had had very bad pustular acne on the face and shoulders for many years. She had been having staphylococcic inoculations at intervals of ten days for about a year. The condition had improved somewhat at first but for several months had been almost stationary. She then had the same stock staphylococcus vaccine, combined with 20,000,000 of her own acne bacillus vaccine. This was followed by the appearance of fresh pustules and the aggravation of existing ones. Ten days afterwards another inoculation was given of staphylococcus, with this time only 10,000,000 acne bacillus, and inoculations were given regularly of the same doses of the same staphylococcic vaccine as she had previously been having, combined with doses of acne bacillus varying from 5,000,000 to 10,000,000 and in three months almost all traces of acne had disappeared. Later she discontinued treatment and a slight relapse occurred, but this has again improved with the mixed staphylococcic and acne bacillus vaccine.

Another case was that of a girl, about 26 years of age, who had had very bad pustular acne on the face for several years. The face was covered with deep indolent pustules; a film

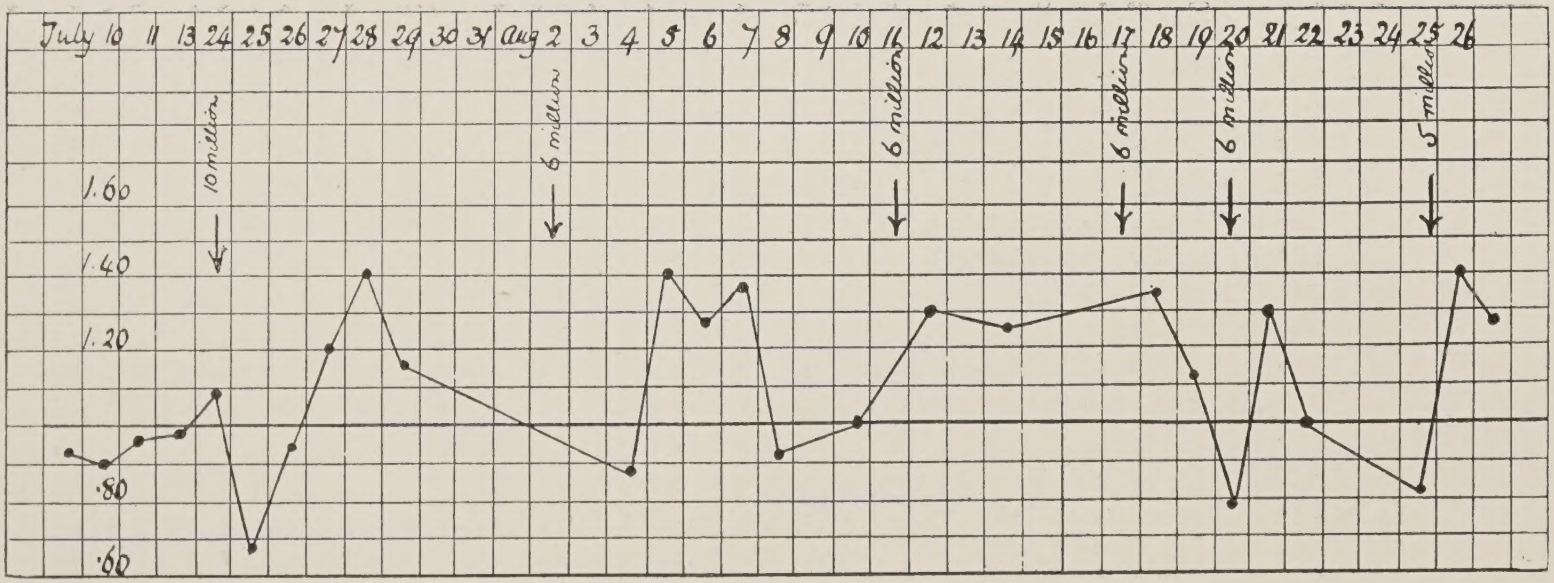

Chart showing the effect on the opsonic index to the acne bacillus produced by inoculations with a vaccine of that organism.

of pus showed no organisms; and of cultures from four pustules two were sterile and two showed a scanty growth of staphylococcus. From July till the end of September last year she had doses of mixed stock staphylococcus and acne bacillus vaccines in doses of 100,000,000 to 250,000,000 of the former and from 5,000,000 to 20,000,000 of the latter, with hardly any improvement. It is worthy of note that while a dose of 250,000,000 staphylococci + 10,000,000 acne bacilli produced hardly any negative phase, the next dose of 250,000,000 staphylococci + 20,000,000 bacilli was followed by a marked exacerbation of the pustulation, indicating the importance of the bacillus in the causation of these lesions. From the end of September till the beginning of January she had the same doses of staphylococcus with doses of from 5,000,000 to 10,000,000 of an acne bacillus obtained in pure culture from her own pustules, and from the very first her condition improved till on Jan. 7th of this year all suppuration had disappeared from the face with the exception of one very small superficial pustule.

Another case was that of a girl, aged 20 years, who had had very bad facial acne for five years. Films of pus showed many acne bacilli and in some pustules staphylococci were also to be seen. From Sept. 3rd, 1908, to Dec. 3rd she was treated with stock vaccines of staphylococcus (200,000,000) and acne bacillus (5,000,000 to 8,000,000) without showing any definite improvement. From Dec. 3rd to Jan. 22nd, 1909, she has been having the same doses of the same staphylococcus with 8,000,000 of an acne bacillus derived from her own lesions. The improvement was immediate and marked, and now there is practically no pustulation, merely some indurated nodules and many scars.

These cases illustrate the fact that not all the cases will show improvement when treated with a stock vaccine, and this is exactly in accordance with what one finds with other organisms, such as streptococcus or bacillus coli. They also furnish very strong evidence that the bacillus is largely responsible for the suppuration. The first case had been treated with staphylococcus for months, and it was only after acne bacillus vaccine was administered that a rapid improvement in the pustulation was observed. The first and second of these cases show that when an excessive dose is given them a definite negative phase is induced, although the dose of staphylococcus is unaltered. The second and third cases

show that although the stock vaccine of staphylococcus combined with the stock vaccines of acne bacillus was of little avail, yet the same doses of the same stock vaccine of staphylococcus with an autogenous acne bacillus vaccine rapidly got rid of the suppuration.

While these cases illustrate the fact that in some cases an autogenous vaccine of acne bacillus is necessary, yet experience has shown that in the vast majority of cases great improvement has been induced by the inoculation of a stock vaccine combined with staphylococcus if an examination of films has revealed that that organism is present. The dose used has varied from 4,000,000 to 10,000,000 and the interval between inoculations from one to two weeks. The guide to treatment has been the appearance of fresh lesions either during the period of low resistance following the positive phase when too long an interval has been allowed to elapse or in the next two or three days after an inoculation indicating that too large a dose has been administered. By watching these signs and working the dose up till it just fails to show any "negative phase" clinically one obtains the maximum benefit from the vaccine. In this way a large number of cases of acne in all its stages have been greatly improved and in a fair proportion the lesions have totally disappeared. On cessation of treatment in several instances, especially in some cases where attendance ceased before the condition had entirely disappeared, there was a recrudescence of the disease, which, however, rapidly gave way to subsequent treatment.

It is interesting in view of Sabouraud's contention that this bacillus is the cause of seborrhœa and of alopecia areata to note the effect of treatment on a patient—a woman aged 25 years—who suffered from comedones on the face and a rapid loss of hair which had been persisting for some months in spite of ordinary treatment. In October last she was first inoculated with the acne bacillus and from the first week after this the loss of hair practically ceased. Since that time she has had inoculations every 7 or 14 days, and there has been no return of the loss of hair. One cannot argue from one case, but it is possible that if, as Sabouraud maintains, seborrhœa of the scalp is due to this bacillus and the loss of hair is due to seborrhœa, then inoculations with the bacillus may prove of benefit in checking this loss.